Genetic Relationships among Species of *Meretrix* (Mollusca: Veneridae) in the Western Pacific Ocean[1]

Ayako Yashiki Yamakawa,[2,3,6] *Masashi Yamaguchi*,[4,5] *and Hideyuki Imai*[4]

Abstract: We compared allozymes at 12 loci in 12 populations of six species of *Meretrix: M. lusoria* (Japan, Korea, and Taiwan), *M. petechialis* (China and Korea), *M. ovum* (Thailand and Mozambique), *M. lyrata* (China), *M. lamarckii* (Japan), and *Meretrix* sp. A (Okinawa, Japan). Our allozyme results were generally consistent with the major groupings currently recognized within the genus based on morphological characters. However, we found two cryptic or undescribed species: *Meretrix* sp. A from Okinawa and *M.* cf. *lusoria* from Taiwan. The shell characters of *Meretrix* sp. A were similar to those of *M. lamarckii*, but the species was genetically distinct (Nei's genetic distance $D > 0.845$) from all other species examined. The Taiwanese *Meretrix* population was morphologically indistinguishable from Japanese *M. lusoria*, although the genetic distance between the Taiwanese and Japanese populations showed a high degree of genetic differentiation ($D > 0.386$). *Meretrix lusoria* seedlings were introduced into Taiwan from Japan in the 1920s, and Japanese *M. lusoria* was previously thought to be established as a cultured stock. However, our results suggest that the Taiwanese population may represent a sibling or cryptic species of *M. lusoria*.

ASIAN HARD CLAMS, genus *Meretrix* (Veneridae), are commercially important bivalves in East and Southeast Asia and East Africa (Yoosukh and Matsukuma 2001). These clams inhabit the tidal flats, estuaries, and sandy beaches of the Indian Ocean, including East Africa and Southeast Asia, and the western Pacific along the Chinese coast, Korean Peninsula, and Japanese Archipelago. The genus comprises nine recognized species: *M. meretrix* (Linnaeus, 1758), *M. casta* (Chemnitz, 1782), *M. lusoria* (Röding, 1798), *M. petechialis* (Lamarck, 1818), *M. ovum* (Hanley, 1845), *M. planisulcata* (Sowerby, 1851), *M. lyrata* (Sowerby, 1851), *M. lamarckii* Gray, 1853, and *M. attenuata* Dunker, 1862 (OBIS Indo-Pacific Molluscan Database 2006). Most are important fishery resources. People have eaten Asian hard clams since ancient times, and *Meretrix* shells are one of the most abundant mollusks found in shell middens in Japan and the Middle East (Kanamaru 1932, Charpentier et al. 2004).

Because of the economic importance of *Meretrix*, previous work has focused mainly on its aquaculture (Yoshida 1941, Wu and Liu 1992, Tuan and Phung 1998), organotin compounds (Midorikawa et al. 2004, Harino et al. 2006), and shellfish poisoning (Nguyen et al. 2006). Seedlings of a few *Meretrix* species are mass-produced, and nearly all hard

[1] Financial support was provided from the 21st Century Center of Excellence (COE) Program of the University of the Ryukyus; the TaKaRa Harmonist Fund by the Takara Shuzo. Co., Ltd.; the Sasakawa Scientific Research Grant by The Japan Science Society; the Mikimoto Fund for Marine Ecology by the Mikimoto & Co., Ltd.; and a research grant by the Interdisciplinary Research Institute of Environmental Sciences. Travel funding was provided from a graduate student research grant by the University of the Ryukyus Foundation. Manuscript accepted 4 September 2007.

[2] Graduate School of Engineering and Science, University of the Ryukyus, Nishihara, Okinawa, 903-0213, Japan.

[3] Department of Regional Economics and Environmental Policy, Okinawa International University, 2-6-1 Ginowan, Ginowan, Okinawa, 901-2701, Japan.

[4] Faculty of Science, University of the Ryukyus, Nishihara, Okinawa, 903-0213, Japan.

[5] Current address: 9658-2 Shiomi, Hyuga, Miyazaki 883-0033, Japan.

[6] Corresponding author (e-mail: a.yamakawa@okiu.ac.jp).

Pacific Science (2008), vol. 62, no. 3:385–394
© 2008 by University of Hawai'i Press
All rights reserved

clams in Taiwanese markets are cultured (Wu and Liu 1989).

Past taxonomic studies of *Meretrix* considered only shell morphology (Fischer-Piette and Fischer 1940–1941, Yoosukh and Matsukuma 2001), although shell shape and color patterns often show marked intraspecific variability (Hamai 1934, 1935, Kosuge 2006). Systematic descriptions of *Meretrix* species are often confusing, and the specific name *M. meretrix* has apparently been used for various species (Yoosukh and Matsukuma 2001). Moreover, the shell form and color of *M. lusoria* and *M. petechialis* are very similar, leading to many erroneous identifications and notations in shell books, reports, and references.

Mitochondrial DNA (mtDNA) has been widely studied in several groups of animals, mainly for taxonomic and phylogenetic purposes. The advantages of using mtDNA include its simple maternal inheritance, absence of recombination, and high substitution rates (Wolstenholme 1992). However, a very high level of gender-associated mtDNA heteroplasmy has been detected in a few bivalve mollusks of the families Mytilidae, Unioidae, and Veneridae (Hoeh et al. 1996, Liu et al. 1996, Beagley et al. 1997, Passamonti and Scali 2001). Thus, the use of mtDNA to estimate evolutionary relationships in bivalves requires great care. Furthermore, the number of loci available for mtDNA analysis is limited. In contrast, allozyme analysis can include a number of loci coded in nuclear DNA simultaneously. Allozyme analysis remains an effective molecular tool with which to investigate phylogenetic relationships and has been much used in phylogenetic and population genetic studies of mollusks (Benzie and Williams 1998, Marins and Levy 1999, Ríos et al. 2002, Inness-Campbell et al. 2003, Väinölä 2003, Martínez et al. 2005, Zaslavskaya 2006).

Despite the economic importance of *Meretrix*, few phylogenetic and population genetic studies of the genus have been undertaken. Molecular techniques can aid in understanding the taxonomy and relationships within this genus. Therefore, we investigated the genetic relationships among *Meretrix* species using electrophoretically detectable allozyme variation.

MATERIALS AND METHODS

Samples

A total of 12 local samples of *Meretrix* spp. was collected in East and Southeast Asia and East Africa in 2005. These clam samples were identified based on shell morphology and coloration. Individuals were sampled from the following locations: *M. lusoria* from Mutsu Bay (32 individuals examined) and Ariake Sea (30), Japan (MluJ-M and MluJ-A, respectively); *M.* cf. *lusoria* from Korea (32) and Taiwan (30) (McfK and McfT, respectively); *M. petechialis* from northern China (30) and Korea (31) (MpC and MpK, respectively); *M. ovum* from Mozambique (32) and Thailand (26) (MoM and MoT, respectively); *M. lyrata* from southern China (25) (MlyC); *M. lamarckii* from Miyagi (32) and Shimane (32), Japan (MlaJ-M and MlaJ-S, respectively); and *Meretrix* sp. A from Okinawa, Japan (23) (MspJ-O). The foot muscle and hepatopancreas were dissected from fresh specimens and immediately frozen at −40°C.

Allozyme Electrophoresis

Horizontal starch-gel electrophoresis was carried out based on the methods of Harris and Hopkinson (1976) and May et al. (1979). Tissue fragments were homogenized with sterilized distilled water. Hydrolyzed starch gels (12.5%) were run at constant voltage. A total of 12 loci from 12 enzymes that showed adequate activity and resolution were routinely examined (Table 1). Terminology and notation of the allozymes were based on recommendations by Shaklee et al. (1990). Two buffer systems were assessed: the Tris-citrate pH 7.0 buffer system (CT-7; gel:buffer dilution 1:10) was run at 250 V for 5 hr for six enzymes, and the citrate-N-(3-aminopropyl) morphoine pH 6.0 buffer system (CAPM-6; gel:buffer dilution 1:4) was run at 250 V for 9 hr for six enzymes. All zymograms were visualized using enzyme-specific stains following recipes in Numachi (1989). Alleles at

TABLE 1

Resolved Enzymes, Most Effective Buffer Systems, and Tissues Used for Allozyme Analyses

Enzyme	Abbreviation	E.C.[a]	Buffer	Tissue[b]
Aspartate aminotranseferase	AAT	2.6.1.1	CAPM-6	F
Aconitase hydrogenase	ACO	4.2.1.3	CAPM-6	H
Catalase	CAT	1.11.1.6	CT-7	H
Glyceraldehyde-3-phosphate dehydrogenase	GAPDH	1.2.1.12	CAPM-6	F
Glucose-6-phosphate isomerase	GPI	5.3.1.9	CT-7	F
Isocitrate dehydrogenase ($NADP^+$)	IDH	1.1.1.42	CT-7	H
Malate dehydrogenase	MDH	1.1.1.37	CAPM-6	F
Malic enzyme ($NADP^+$)	ME	1.1.1.40	CAPM-6	F
Peptidase using leucyl-glycyl-glycine substrate	PEP-lgg	3.4.11–13	CT-7	H
6-Phosphogluconate dehydrogenase	6PGD	1.1.1.44	CT-7	H
Phosphoglucomutase	PGM	2.7.5.1	CT-7	F
Superoxidase dismutase	SOD	1.15.1.1	CAPM-6	H

[a] Enzyme commission number.
[b] F, foot; H, hepatopancreas.

each locus were labeled alphabetically in order of the relative electrophoretic mobility of the allozymes.

Data Analysis

Genotype and allele frequencies for the 12 loci were determined for all local samples. Genotype frequencies at polymorphic loci were examined for agreement with the expectations of Hardy-Weinberg equilibrium using a chi-square test. The mean biased estimate of expected heterozygosity (H_e), mean observed heterozygosity (H_o), and percentage of polymorphic loci (P) within samples, as well as Nei's (1978) unbiased measures of genetic identity (I) and genetic distance (D) between samples, were calculated using POPGENE v. 1.32 (Yeh et al. 1999). The unweighted paired group method of cluster analysis (UPGMA [Sokal and Sneath 1963]) was used to construct a phylogenetic tree.

RESULTS

Allele frequencies at the 12 polymorphic loci and average measures of genetic variation of all samples are presented in Table 2. The levels of genetic variation differed considerably among the samples, with average expected heterozygosity (*He*) varying from 0.090 to 0.375 (Table 2). Several interspecific diagnostic alleles were observed: allele F and G at the *ACO* locus in *M. ovum*, allele A at the *GPI* locus in *M. lyrata*, the combination of allele A and C at the *MDH-2* locus and allele B and C at the *SOD-2* locus in *M. lamarckii*, and the combination of allele A at the *IDH* locus and allele C at the *MDH-2* locus in *Meretrix* sp. A (Table 2). However, no diagnostic alleles were observed for *M. lusoria*, *M.* cf. *lusoria*, or *M. petechialis*.

Unbiased genetic identities (I) (Nei 1978) between taxa varied from 0.162 between MluJ-A and MoT to 0.850 between McfK and MpK (Table 3). Genetic identities between separated conspecific populations within taxa were 0.970 (MluJ-M and MluJ-A), 0.976 (MpC and MpK), 0.818 (MoT and MoM), and 0.949 (MlaJ-S and MlaJ-O). Intraspecific identities were generally higher than interspecific identities (Figure 1). However, two modes of interspecific genetic identity were clear: 0.25–0.3 and 0.8–0.85. The genetic identities between *M. lusoria*, *M.* cf. *lusoria*, and *M. petechialis* were quite high, averaging 0.783, indicating that these species are closely related.

Nei's unbiased genetic distance (D) between taxa ranged from 0.163 between McfK and MpK to 1.819 between MluJ-A and MoT (Table 3). The lowest D for all pairwise population comparisons were within taxa: 0.030 between MluJ-M and MluJ-A, 0.025 between

TABLE 2

Allele Frequencies at Twelve Loci for *Meretrix* Species

Species		*M. lusoria*		*M.* cf. *lusoria*		*M. petechialis*		*M. ovum*		*M. lyrata*	*M. lamarckii*		*Meretrix* sp.
Code		MluJ-M	MluJ-A	McfK	McfT	MpC	MpK	MoT	MoM	MlyC	MlaJ-M	MlaJ-S	MspJ-O
Locus	Allele	N = 32	N = 30	N = 30	N = 32	N = 30	N = 31	N = 23	N = 26	N = 32	N = 32	N = 32	N = 25
AAT-2	A			0.016	0.781	0.150	0.065						
	B			0.032	0.031	0.033	0.032						
	C	0.875	1.000	0.871	0.188	0.200	0.355						
	D							0.587			0.500	0.641	
	E	0.031		0.032		0.083	0.016						
	F							0.044		1.000	0.453	0.359	1.000
	G	0.094		0.048		0.533	0.532						
	H							0.370	1.000		0.047		
ACO	A												0.020
	B			0.016									0.880
	C	0.531	0.083	0.016	0.031	0.050	0.065						
	D	0.469	0.917	0.968	0.969	0.950	0.936						0.060
	E									1.000	1.000	1.000	0.040
	F								0.250				
	G							1.000	0.750				
CAT	A										0.250	0.813	
	B	0.078	0.033		0.281	0.017		0.065	0.192		0.750	0.156	0.200
	C	0.156	0.267	0.081	0.359	0.033		0.087	0.308				0.680
	D	0.766	0.700	0.919	0.359	0.950	1.000	0.848	0.500	1.000			0.120
GAPDH	A				0.484	0.150		1.000	1.000		0.969	1.000	
	B												0.800
	C			0.032			0.258				0.031		
	D	1.000	0.983	0.968	0.516	0.850	0.742			1.000			0.200
	E		0.017										
GPI	A									1.000			
	B							0.457			0.984	1.000	
	C	1.000	0.967	0.936	0.297	0.300	0.210						
	D							0.413	0.423		0.016		1.000
	E		0.033	0.065	0.641	0.683	0.790						
	F							0.130	0.577				
	G				0.063	0.017							
IDH	A		0.017					1.000	1.000				1.000
	B	1.000	0.983	1.000	1.000	1.000	1.000			1.000	1.000	1.000	
MDH-2	A								0.096			0.063	
	B			0.097	0.578	0.067	0.048						
	C										1.000	0.938	1.000
	D	1.000	1.000	0.887	0.422	0.933	0.952	0.022	0.712				
	E			0.016				0.978	0.192	0.516			
	F									0.484			

ME	A										0.969	0.906	
	B		0.033			0.033						0.094	
	C	0.938	0.933	0.677		0.333	0.258	1.000	0.981	1.000	0.016		1.000
	D	0.047	0.033	0.307	1.000	0.600	0.742						
	E	0.016		0.016		0.033			0.019		0.016		
PEP-lgg	A										0.766	0.563	
	B							0.130			0.063	0.172	
	C										0.172	0.250	0.320
	D								0.365			0.016	0.660
	E							0.522	0.250				
	F		0.033						0.192	0.813			0.020
	G						0.016						
	H	0.250	0.267	0.403	0.109	0.050		0.217	0.135	0.188			
	I		0.083		0.109		0.150	0.097					
	J	0.531	0.317	0.403	0.547		0.065	0.044	0.058				
	K	0.109	0.083	0.065	0.031	0.417	0.532						
	L							0.087					
	M	0.109	0.150	0.129	0.156	0.333	0.210						
	N		0.067		0.047		0.032						
	O					0.050	0.048						
6PGD	A	0.047	0.050	0.161	0.328	0.617	0.339	0.544	0.308		0.063	0.031	
	B	0.875	0.717	0.742	0.609	0.350	0.645	0.457	0.692	0.094	0.625	0.344	0.980
	C	0.078	0.233	0.097	0.063	0.033	0.016			0.906	0.313	0.625	0.020
PGM	A						0.016						
	B		0.033			0.017	0.016				0.219	0.109	
	C	0.016	0.067	0.113		0.100	0.016						
	D	0.266	0.317	0.371	0.172	0.233	0.323	0.174	0.346		0.281	0.328	
	E	0.094	0.067	0.065	0.188	0.100	0.129			0.031			
	F	0.547	0.383	0.339	0.469	0.217	0.290	0.783	0.385	0.953	0.375	0.375	0.100
	G	0.016	0.017	0.032	0.047	0.017	0.081				0.094	0.172	
	H	0.047	0.083	0.081	0.109	0.317	0.113	0.044	0.269	0.016		0.016	0.880
	I				0.016		0.016				0.031		
	J	0.016	0.033										0.020
SOD-2	A							0.957	0.962				
	B										1.000	0.969	
	C							0.044	0.039			0.031	
	D	1.000	1.000	1.000	1.000	1.000	1.000						1.000
	E									1.000			
H_e		0.229	0.232	0.260	0.375	0.351	0.321	0.257	0.329	0.090	0.227	0.247	0.148
H_o		0.201	0.192	0.191	0.354	0.317	0.266	0.283	0.359	0.089	0.221	0.188	0.143
n_a		2.50	2.83	2.92	2.75	3.17	3.08	2.25	2.25	1.42	2.25	2.25	1.92
n_e		1.47	1.70	1.59	1.90	1.93	1.80	1.54	1.81	1.15	1.52	1.55	1.24
P		58.33	75.00	83.33	75.00	83.33	75.00	66.67	75.00	33.33	66.67	66.67	50.00

Note: n_a, n_e, mean number of alleles per locus and effetive number of alleles, respectively; *P*, proportion of polymorphic loci per population; N, sample size; H_o, H_e, average observed and expected heterozygosities, respectively.

TABLE 3

Matrix of Nei's Genetic Identity (*I*, above Diagonal) and Genetic Distance (*D*, below Diagonal) among 12 Populations of *Meretrix* Species Based on 12 Allozyme Loci

Genus	*Meretrix*											
Species	*lusoria*		cf. *lusoria*		*petechialis*		*ovum*		*lyrata*	*lamarckii*		sp.
Code	MluJ-M	MluJ-A	McfK	McfT	MpC	MpK	MoT	MoM	MlyC	MlaJ-M	MlaJ-S	MspJ-O
MluJ-M		0.970	0.888	0.652	0.729	0.745	0.188	0.254	0.351	0.206	0.191	0.251
MluJ-A	0.030		0.911	0.674	0.760	0.764	0.162	0.237	0.341	0.191	0.189	0.246
McfK	0.119	0.093		0.741	0.845	0.850	0.265	0.320	0.419	0.192	0.176	0.308
McfT	0.427	0.395	0.299		0.809	0.808	0.214	0.259	0.272	0.278	0.246	0.256
MpC	0.316	0.275	0.168	0.212		0.976	0.244	0.292	0.370	0.186	0.176	0.262
MpK	0.295	0.269	0.163	0.213	0.025		0.222	0.276	0.355	0.190	0.171	0.256
MoT	1.670	1.819	1.329	1.544	1.410	1.506		0.818	0.326	0.273	0.267	0.334
MoM	1.369	1.439	1.140	1.351	1.230	1.288	0.201		0.234	0.216	0.181	0.430
MlyC	1.046	1.077	0.870	1.304	0.994	1.035	1.120	1.454		0.314	0.333	0.245
MlaJ-M	1.579	1.653	1.648	1.279	1.684	1.663	1.298	1.531	1.157		0.949	0.244
MlaJ-S	1.658	1.667	1.740	1.404	1.736	1.764	1.319	1.711	1.099	0.053		0.193
MspJ-O	1.384	1.402	1.177	1.364	1.340	1.362	1.098	0.845	1.405	1.409	1.645	

MpC and MpK, 0.201 between MoT and MoM, and 0.053 between MlaJ-M and MlaJ-S. In the UPGMA tree based on genetic distance (Figure 2), *M. lamarckii* (MlaJ-M and MlaJ-S) was the first to diverge from other *Meretrix* species. *Meretrix* sp. A (MspJ-O) clustered with *M. ovum* (MoT and MoM), and *M. lyrata* (MlyC) was within a cluster containing *M. lusoria*, *M.* cf. *lusoria*, and *M. petechialis*. The Korean *M.* cf. *lusoria* McfK population joined with the *M. lusoria* (MluJ-M and MluJ-A) cluster, with *D* of 0.119 between McfK and MluJ-M and *D* of 0.093 between McfK and MluJ-A. In contrast, the Taiwanese *M.* cf. *lusoria* McfT population joined the *M. petechialis* (MpC and MpK) cluster. The *D* between McfT and MpC was 0.212 and that between McfT and MpK was

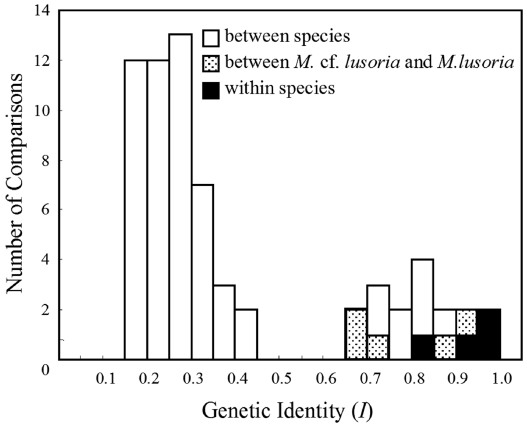

FIGURE 1. Frequency distribution of genetic identity (*I*) based on 12 allozyme loci within species (i.e., *M. lusoria*, *M. ovum*, and *M. lamarckii*), between *M.* cf. *lusoria* and *M. lusoria*, and between *Meretrix* species.

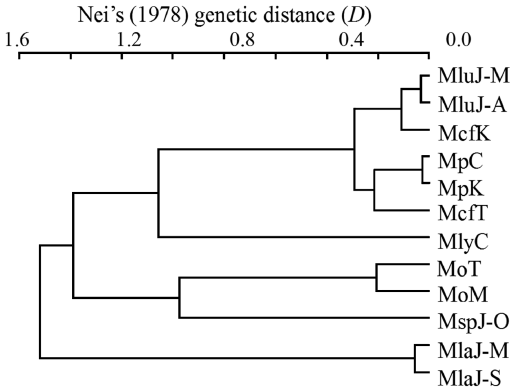

FIGURE 2. UPGMA tree of Nei's (1978) genetic distance, showing the genetic relationships among 12 populations of *Meretrix* species based on 12 allozyme loci.

0.213. Within the genus *Meretrix*, *M. lusoria* and *M. petechialis* were the most closely related species, with a small genetic distance ($D < 0.316$).

DISCUSSION

The results of our allozyme analysis were largely consistent with the major groupings currently recognized within the genus *Meretrix* based on morphological characters, except for two species: *Meretrix* sp. A (MspJ-O) from Okinawa and *M.* cf. *lusoria* from Taiwan (McfT) (Figure 2). *Meretrix* sp. A (MspJ-O) is a sister taxon to *M. ovum*, although its shell morphology and habitat preference are similar to those of *M. lamarckii;* indeed, Kosuge (2003) identified the Okinawan species as *M. lamarckii*. Both species prefer dissipative sandy beaches facing high-salinity open seas and share similar morphological adaptations such as a relatively rectilinear ventral margin and a deeper pallial sinus than *M. lusoria*, *M. petechialis*, *M. lyrata*, and *M. ovum*. However, the genetic data indicated a marked difference in that *Meretrix* sp. A (MspJ-O) was genetically distant from all the other species examined ($D > 0.845$). Because this species does not, to our knowledge, morphologically or genetically match any other known species, it may be an undescribed *Meretrix* species.

Meretrix lusoria seedlings were introduced to Taiwan from Japan in 1925 and have spread widely in many sandy beaches and estuaries on the west coast of Taiwan (Chen 1984, Chien and Hsu 2006). Hard-clam aquaculture expanded rapidly, with the percentage of aquaculture-produced clams on the market reaching 98.8% in 1986 (Wu and Liu 1989). Introduced Japanese *M. lusoria* was assumed to have been established as a cultured stock in Taiwan, and cultured hard clams were thought to be an introduced species. However, the Taiwanese population (McfT) shows a high degree of differentiation ($D > 0.395$ [Table 3]) from Japanese *M. lusoria* populations (MluJ-M and MluJ-A [Figure 2]). At the *AAT-2*, *GAPDH*, *GPI*, and *Me* loci, Japanese *M. lusoria* populations and Taiwanese *M.* cf. *lusoria* showed limited allele

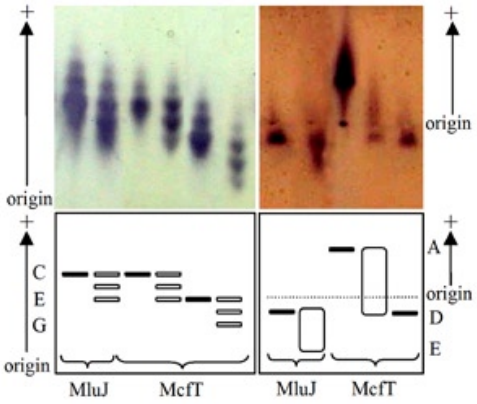

FIGURE 3. Electrophoretic patterns of Japanese *M. lusoria* (MluJ-M and MluJ-A) and Taiwanese *M.* cf. *lusoria* (McfT). *Left: GPI* locus (Dimer). *Right: GAPDH* locus (Tetramer).

frequency overlap (3–50% [Table 2 and Figure 3]). This suggests that McfT and *M. lusoria* may be different species. The shell morphology and coloration of *M. lusoria* and the Taiwanese population (McfT) do not differ clearly, suggesting that the Taiwanese population may be a cryptic species of *M. lusoria*. Our results suggest that a local Taiwanese *Meretrix* existed before the introduction of Japanese *M. lusoria* in 1925 and that Japanese *M. lusoria* might have failed to survive as a cultured stock because of habitat or climate differences.

Within the genus *Meretrix*, *M. lusoria* and *M. petechialis* were the most closely related, with small genetic distances ($D < 0.316$ [Table 3]). In line with this genetic closeness, *M. lusoria* and *M. petechialis* are similar in morphological characters and are occasionally confused in illustrated books and references.

Some Korean *M.* cf. *lusoria* (McfK) individuals showed morphological characteristics of both *M. lusoria* and *M. petechialis*. Nevertheless, our genetic data indicate that Korean McfK belongs to *M. lusoria*. In the Asian continent, the habitat of *M. lusoria* is limited to the southern part of the Korean Peninsula (Yamashita et al. 2004), whereas *M. petechialis* occurs from the west coast of the Korean Peninsula to southern China and Vietnam.

The habitat preferences of *M. lusoria* and *M. petechialis* appear very similar: both occur in sandy and muddy tidal flats in estuarine environments of inland seas. In South Korea, the natural distribution of *M. lusoria* and *M. petechialis* is allopatric, which probably maintains reproductive isolation between these two species.

In Japan, *M. petechialis* seedlings imported from the Asian continent have been released into the natural habitat of *M. lusoria* since the 1990s (Japanese Ministry of the Environment 2005). If the reproductive isolation of *M. lusoria* and *M. petechialis* was not complete, hybridization between these two species could occur because of the artificial sympatric distribution in Japan. Kawase (2002) claimed that hybrids between the local *M. lusoria* and introduced *M. petechialis*, diagnosed by morphological characters, were found in Aichi Prefecture, Japan. Further investigation using DNA is needed to confirm the existence of hybrid individuals.

We analyzed two unknown *Meretrix* species (i.e., McfT and MspJ-O) in addition to five of the nine described species (i.e., *M. lusoria*, *M. petechialis*, *M. ovum*, *M. lyrata*, and *M. lamarckii*). Future combined morphological and genetic investigations may reveal additional undescribed *Meretrix* species.

Meretrix species are distributed broadly in the West Pacific, Asia, and the Indian Ocean. The center of origin of *Meretrix* seems to be located in the Indo-Malayan subregion of the Indo-West Pacific. *Meretrix ovum* is widely distributed compared with other *Meretrix* species: it inhabits muddy bottoms from the Mekong River in Vietnam and Gulf of Thailand (Yoosukh and Matsukuma 2001) to Mozambique. We hypothesize that the ancestral species of *Meretrix* may be closely related to *M. ovum* and may have dispersed in two directions (i.e., west and northeast) from the Indo-Malayan subregion by means of larval dispersal along strong ocean currents. During the process of spreading, the ancestral species may have diverged into two major types (i.e., mud-flat species in estuarine environments and sandy-beach species in open sea areas). Further studies that include the species not examined here and use genetic sequence data, particularly from DNA, may resolve these phylogeographic issues.

ACKNOWLEDGMENTS

We are grateful to Prof. T. Y. Shy and Ms. K. Lee, National Penghu University; Mr. J. Y. Kao, Noah's Ark Ecological Engineering Consultant Co., Ltd., Taiwan; Mr. A. Endo, Ichi Lda., Mozambique and Ms. S. Toda, Japan International Cooperation Center; and A. Inouye and S. Lee, University of the Ryukyus, for their help in collecting samples. We also thank Dr. H. Saito, National Science Museum; Dr. B. Metivier, Muséum National d'Histoire Naturelle; and Dr. Y. Finet, Muséum d'Histoire Naturelle de la Ville de Genève, for their support in observing *Meretrix* specimens in museum collections. Our heartfelt thanks go to Dr. K. Numachi for editorial comments. Finally, we thank Prof. Y. Ikeda, Prof. S. Suda, Mr. T. Yoshino, Dr. K. Tachihara, and all of our laboratory members at the University of the Ryukyus for their help and comments.

Literature Cited

Beagley, C. T., K. A. Taylor, and D. R. Wolstenholme. 1997. Gender-associated diverse mitochondrial DNA molecules of the mussel *Mytilus californianus*. Curr. Genet. 31:318–324.

Benzie, J. A. H., and S. T. Williams. 1998. Phylogenetic relationships among giant clam species (Mollusca: Tridacnidae) determined by protein electrophoresis. Mar. Biol. (Berl.) 132:123–133.

Charpentier, V., S. Méry, and C. Phillips. 2004. Des coquillages … outillages des Ichtyophages? Mise en évidence d'industries sur Veneridae, du Néolithique à l'âge du Fer (Yémen, Oman, E.A.U). Arab. Arch. Epig. 15:1–10 [in French with English abstract].

Chen, H. C. 1984. Recent innovations in cultivation of edible molluscs in Taiwan, with special reference to the small abalone *Haliotis diversicolor* and the hard clam *Meretrix lusoria*. Aquaculture 39:11–27.

Chien, Y. H., and W. H. Hsu. 2006. Effects of diets, their concentrations and clam size

on filtration rate of hard clams (*Meretrix lusoria*). J. Shellfish Res. 25:15–22.

Fischer-Piette, E., and P. H. Fischer. 1940–1941. Révision des espèces vivantes de *Meretrix* s. s. du Muséum National d'Histoire Naturelle. J. Conchyliol. 84:313–345 [in French].

Hamai, I. 1934. On the local variation in the shells of *Meretrix meretrix* (L.), with special reference to growth of organism. Sci. Rep. Tohoku Imp. Univ. Biol. 9:131–158.

———. 1935. A study of one case in which different environmental conditions produce different types of *Meretrix meretrix*. Sci. Rep. Tohoku Imp. Univ. Biol. 10:485–498.

Harino, H., S. Midorikawa, T. Arai, M. Ohji, N. D. Cu, and N. Miyazaki. 2006. Concentrations of booster biocides in sediment and clams from Vietnam. J. Mar. Biol. Assoc. U. K. 86:1163–1170.

Harris, H., and D. A. Hopkinson. 1976. Handbook of enzyme electrophoresis in human genetics. North-Holland, Amsterdam.

Hoeh, W. R., D. T. Stewart, B. W. Sutherland, and E. Zouros. 1996. Multiple origins of gender-associated mitochondrial DNA lineages in bivalves (Mollusca: Bivalvia). Evolution 50:2276–2286.

Inness-Campbell, J., M. Stuckey, and M. S. Johnson. 2003. Allozymic investigation of phylogeny of *Littoraria* (Gastropoda: Littorinidae). J. Molluscan Stud. 69:19–26.

Japanese Ministry of the Environment. 2005. Information on *Meretrix petechialis*. Wildlife Division, Nature Conservation Bureau (http://www.env.go.jp/nature/intro/1outline/caution/detail_mu.html#11).

Kanamaru, T. 1932. Japanese hard clam story in Ise. Venus 3 (3): 144–154 [in Japanese].

Kawase, M. 2002. Macrobenthic organisms in the estuary of the Yahagi River. Yahagigawakenkyu 6:81–98 [in Japanese].

Kosuge, T. 2003. Occurrence of *Meretrix lamarckii* Deshayes (Mollusca, Bivalvia, Veneridae) at a sandy beach near the mouth of Urauchi River, Iriomote Island, Okinawa, Japan. Nankiseibutsu 45 (2): 128–131 [in Japanese].

———. 2006. Notes on the lyrate hard clam *Meretrix* sp. (Bivalvia: Veneridae) in Vietnam, with special reference to its introduction to the northern coasts of Vietnam. Chiribotan 36 (4): 132–135 [in Japanese].

Liu, H., J. B. Mitton, and S. Wu. 1996. Paternal mitochondrial DNA differentiation far exceeds maternal mitochondrial DNA and allozyme differentiation in the freshwater mussel, *Anodonta grandis*. Evolution 50:952–957.

Marins, L. F., and J. A. Levy. 1999. High genetic distance between marine bivalves of the genus *Mesodesma* inhabiting the Atlantic and Pacific coasts of South America. Comp. Biochem. Physiol. A Comp. Physiol. 124:313–319.

Martínez, P., M. Pérez-Losada, A. Guerra, and A. Sanjuan. 2005. First genetic variation and diagnosis of the short-finned squid species of the genus *Illex* (Cephalopoda: Ommastrephidae). Mar. Biol. (Berl.) 148:97–108.

May, B., J. E. Wright, and M. Stoneking. 1979. Joint segregation of biochemical loci in Salmonidae: Results from experiments with *Salvelinus* and review of the literature on other species. J. Fish. Res. Board Can. 36:1114–1128.

Midorikawa, S., T. Arai, H. Harino, N. D. Cu, P. A. Duc, and N. Miyazaki. 2004. Organotin levels in bivalves in Southeast Asia. Coastal Mar. Sci. 29 (1): 57–62.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583–590.

Nguyen, V. N., D. V. Ha, and L. T. Tung. 2006. *Dinophysis* spp. recorded in the coastal waters of northern Vietnam during 2002–2003. Coastal Mar. Sci. 30 (1): 107–110.

Numachi, K. 1989. Population differentiation of marine organisms by isozyme analysis. Pages 42–63 *in* Japan Fisheries Conservation Association, ed. Report on the genetic assessment project. Japan Fisheries Resource Conservation Association, Tokyo [in Japanese].

OBIS Indo-Pacific Molluscan Database. 2006. (http://data.acnatsci.org/obis/find_mollusk.html).

Passamonti, M., and V. Scali. 2001. Gender-associated mitochondrial DNA heteroplasmy in the venerid clam *Tapes philippinarum* (Mollusca: Bivalvia). Curr. Genet. 39:117–124.

Ríos, C., S. Sanz, C. Saavedra, and J. B. Pena. 2002. Allozyme variation in populations of scallops, *Pecten jacobaeus* (L.) and *P. maximus* (L.) (Bivalvia: Pectinidae), across the Almeria-Oran front. J. Exp. Mar. Biol. Ecol. 267:223–244.

Shaklee, J. B., F. W. Allendorf, D. C. Morizot, and G. S. Whitt. 1990. Gene nomenclature for protein-coding loci in fish. Trans. Am. Fish. Soc. 119:2–15.

Sokal, R. R., and P. H. A. Sneath. 1963. Principles of numerical taxonomy. Freeman, San Francisco.

Tuan, V. S., and N. H. Phung. 1998. Status of bivalve exploitation and farming in the coastal waters of South Vietnam. Spec. Publ. Phuket Mar. Biol. Cent. 18 (1): 171–174.

Väinölä, R. 2003. Repeated trans-Arctic invasions in littoral bivalves: Molecular zoogeography of the *Macoma balthica* complex. Mar. Biol. (Berl.) 143:935–946.

Wolstenholme, D. R. 1992. Animal mitochondrial DNA: Structure and evolution. Int. Rev. Cytol. 141:173–216.

Wu, W. L., and H. P. Liu. 1989. Malacological research on *Meretrix* resources in Taiwan II. History review and evaluation on the studies of the Taiwan *Meretrix*. Bull. Malacol. Rep. China 14:49–61 [in Chinese with English abstract].

———. 1992. Developmental rhythm on gamete and gonad of *Meretrix lusoria* from Taiwan (Bivalvia: Veneridae). Bull. Malacol. Rep. China 17:79–86 [in Chinese with English abstract].

Yamashita, H., S. Sato, K. W. Kim, Y. Henmi, H. Nagata, S. Yamamoto, A. Ikeguchi, Y. Mizuma, J. Nawa, and R. Takashima. 2004. The silent mud flat. Report of the Takagi Fund for Citizen Science 1:85–91 [in Japanese].

Yeh, F. C., R. C. Yang, T. Boyle, Z. H. Ye, and J. X. Mao. 1999. POPGENE, version 1.31, the user-friendly shareware for population genetic analysis. Molecular Biology and Biotechnology Centre, University of Alberta, Canada.

Yoosukh, W., and A. Matsukuma. 2001. Taxonomic study on *Meretrix* (Mollusca: Bivalvia) from Thailand. Spec. Publ. Phuket Mar. Biol. Cent. 25 (2): 451–460.

Yoshida, H. 1941. Notes on the early life-history of *Meretrix meretrix*. Venus 11 (1): 1–11 [in Japanese with English summary].

Zaslavskaya, N. I. 2006. Allozyme comparison of *Littorina* species from the northwestern Pacific. J. Molluscan Stud. 72:163–166.